Localization of the Alpha 3 (V) Chain of Type V Collagen in Human Skin

David T. Woodley, M.D., Vicki J. Scheidt, D.V.M., Melinda J. Reese, B.S., Amy S. Paller, M.D., Thomas O. Manning, D.V.M., Takashi Yoshiike, M.D., and Robert A. Briggaman, M.D.
Department of Dermatology, University of North Carolina School of Medicine, Chapel Hill, North Carolina, U.S.A.

Serum from goats immunized with human type V collagen chains that were cut out of polyacrylamide gels contained an antibody that recognized only type V collagen in an enzyme-linked immunoabsorbent assay and did not label laminin, fibronectin, or types I and IV collagen. Western blot analysis of the antibody showed that its determinant was the alpha 3 (V) chain of type V collagen. Indirect immunofluorescent staining of intact human skin with the antibody produced staining of the dermal blood vessels but not of the dermal-epidermal junction (DEJ). In contrast, both the dermal blood vessels and the DEJ were labeled by the antibody if the skin substrate was first split through the lamina lucida region of the DEJ by incubation in 1 M NaCl solution. Indirect immunoelectron microscopy confirmed the staining pattern found by immunofluorescence and defined the ultrastructural localization of type V collagen in skin. Type V collagen is localized within the DEJ to the lamina lucida region and polar aspects of the basal cell keratinocyte plasma membrane. *J Invest Dermatol 88: 246–252, 1987*

Type V collagen was first isolated from human placenta by Burgeson et al [1] and Chung, Rhodes, and Miller [2]. When placental villi are included in the preparations in addition to fetal membranes, type V collagen consists of three distinct alpha chains in a trimer of alpha 1 (V), alpha 2 (V), and alpha 3 (V)—so-called ABC trimers [3,4]. When analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), three characteristic chains are revealed and denoted as A, B, and C, or alpha 2 (V), alpha 1 (V), and alpha 3 (V), respectively. The C chain (alpha 3 [V]) is not found in all tissues such as amnion and may be a minor component on the type V molecule. In addition to alpha chains, biosynthesis studies have demonstrated large procollagen chains (M_r = 239K and M_r = 174K). Significant portions of the nonhelical propeptide segments are retained in the tissue forms of type V collagen, a feature similar to type IV collagen and distinct from the interstitial collagens. Further, type V collagen has a characteristically low alanine content of 40 residues per 1000 amino acids [1].

Type V collagen is distributed in many tissues as diverse as bone, tendon, kidney, muscle, lung, and skin. Although well characterized biochemically, its localization within tissues is variable; pericellular, interstitial, and basement membrane localizations have been reported. Duance and associates [5,6] found type V collagen associated with basement membranes. Likewise, Roll et al [7], using immunoelectron microscopy, found that type V collagen was within basement membranes and codistributed with type IV collagen. Using similar immunoelectron microscopic techniques as Roll and coworkers [7], Gay et al [8] could not confirm that type V collagen was within basement membranes. Their studies and those of others [9,10] showed that type V collagen had a pericellular localization around many cell types, including smooth muscles and endothelial cells. In the human amnion, type V collagen was found within basement membrane using a polyclonal anti-type V collagen antibody raised in NZW rabbits against human placental type V collagen [4]. This antibody labeled 12-nm unbanded fibrils that extended from the lamina densa into the interstitial connective tissue. Further, the type V unbanded fibrils were frequently enmeshed among the type I collagen fibers in the interstitium abutting the basement membrane zone (BMZ). An interstitial distribution by light microscopy also was reported by Linsenmayer et al [11] using a monoclonal anti-type V collagen antibody. Except for the above-noted report of 12-nm fibrils in the pericellular interstitium of amnion, the morphology of tissue forms of type V collagen remains uncertain. Although these apparently discrepant results might be explained by technical variations, it is also possible that type V collagen may be polymorphic and multifunctional in different tissues or in different locations in the same tissue.

In skin, the localization of type V collagen is also controversial. Stenn, Madri, and Roll [12] supported the cutaneous localization of antibodies to type V collagen in mouse dermal-epidermal junction (DEJ). However, Gay et al [8] could not demonstrate type V collagen in human skin DEJ using antibodies specific for type V collagen. However, this latter group showed that antibodies to the C chain of type V collagen stained the BMZ of normal and psoriatic skin [13]. Ultrastructurally, these antibodies labeled the lamina densa region. Using an affinity-purified anti-type V collagen antibody prepared in rats and immunofluorescent stain-

Manuscript received April 15, 1986; accepted for publication August 26, 1986.

This work was supported by National Institutes of Health grants AM33625, AM01540, and a grant from the American Cancer Society.

Reprint requests to: David T. Woodley, M.D., Department of Dermatology, North Carolina Memorial Hospital, Room 137, Chapel Hill, North Carolina 27514.

Abbreviations:
BMZ: basement membrane zone
DEJ: dermal-epidermal junction
EBA: epidermolysis bullosa acquisita
ELISA: enzyme-linked immunoabsorbent assay
IIF: indirect immunofluorescence
PBS: phosphate-buffered saline
SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis

0022-202X/87/$03.50

ing, Konomi et al [14] found type V collagen located adjacent to cutaneous basement membranes. However, the ultrastructural localization was not reported. In other studies, immunofluorescent staining of intact human skin and human skin split at the DEJ by various means showed that type V collagen was localized to dermal blood vessels and, in one case, to the DEJ [15] using polyclonal anti-type V collagen antibody. Ultrastructural localization was not described.

One possible explanation for the discrepancies reported in the literature is that the epitopes on the type V collagen molecule to which the antibodies are directed have not been demonstrated. In this study, we show that antibodies to type V collagen alpha 3 chain (C chain) bind to the BMZ around dermal blood vessels as well as to the BMZ within the DEJ. The binding of this antibody within the DEJ occurred only when the junction was separated through the lamina lucida by pretreatment with 1 M sodium chloride solution. After the junction was separated, type V collagen was revealed within the lamina lucida of the basement membrane and apposed pericellularly to the plasma membrane of the basal keratinocytes.

MATERIALS AND METHODS

Preparation of Type V Collagen Human placentas were obtained fresh from the delivery suite at North Carolina Memorial Hospital and kept at 4°C. Type V collagen was isolated from the placentas by acid extraction and sequential salt precipitation as described by Sage and Bornstein [16]. Contaminating type I collagen was removed from the final extract by carboxymethyl (CM) cellulose ion exchange chromatography. The column was equilibrated in 2.0 M urea, 0.04 M lithium acetate, and 0.03 M lithium chloride, pH 4.8, and developed in the same buffer with a superimposed linear gradient of sodium chloride from 0.03 to 0.25 M at room temperature as described by Burgeson et al [1]. The final preparation was subjected to SDS-PAGE [17] and amino acid analysis (performed by Dr. David Klapper, Department of Microbiology, University of North Carolina Medical School).

Type V Collagen Antibodies Fifty micrograms of type V collagen preparation isolated above was electrophoresed in each lane of a 5% SDS-PAGE slab gel and stained with Coomassie Blue as described by Laemmli [17]. The revealed type V collagen chains were cut out of the gels after several washes in distilled water, minced into pieces, and passed in a sequential fashion through the barrel of a syringe, an 18-gauge needle, and a 21-gauge needle. The minced pieces were then mixed 2 parts to 1 part (vol/vol) with complete Freund's adjuvant for initial immunizations and incomplete Freund's adjuvant thereafter and emulsified into a semisolid state by passing the solution back and forth between the Luer-lock connected syringes. For each preparation, 20 lanes containing 50 μg of the type IV collagen were prepared for a total protein content of 1000 μg in a vol of 2–3 ml. The type V collagen/Freund's adjuvant emulsions were injected intradermally into female goats monthly. Fifty to eighty milliliters of blood was obtained 2 weeks after each injection from the goats and the serum separated by centrifugation [18].

Western Blot Analysis of Antibody Serum obtained monthly from immunized goats was analyzed for reactivity against type V collagen by Western blot analysis [19]. Fifty micrograms per lane of type V collagen or type V collagen preparations contaminated with type I collagen was subjected to SDS-PAGE, electrophoretically transferred to nitrocellulose paper, washed in 0.01 M Tris-HCl pH 7.5, 0.25 M NaCl, 0.03% Nonidet P-40 detergent (Buffer A), and incubated with sera diluted 1:200–1:400 in Buffer A with 1% bovine serum albumin. After washing in the same buffer, the strips were incubated in peroxidase-labeled antigoat IgG diluted 1:500 in buffer A for 2 h, washed, and then reacted with 4-chloro-1-naphthol as previously described [20]. The reaction was terminated by washing the strips in cold water several times. The strips were dried on filter paper, mounted, and photographed.

Identical experiments were performed using substrates other than type V collagen. These included epidermal cell proteins derived from suction blister roofs [20], laminin [21], fibronectin [22], bovine serum albumin (Sigma Chemical Co., St. Louis, Missouri), elastin (kindly provided by Dr. Jeff Davidson, University of Utah), types I and IV collagen [21,23], and a human skin BMZ extract [24].

The prepared placental type V collagen was also analyzed by Western blot against antibodies to laminin [21] (BRL, Bethesda, Maryland), fibronectin [22], types I and IV collagen [21], and a monoclonal antibody to the epidermolysis bullosa acquisita (EBA) antigen [25] in dilutions of 1:100.

Two-dimensional gel electrophoresis as described by O'Farrell [26] and modified by Ames and Nikaido [27] was performed on type V collagen preparations and then immunoblotted as described by Koulu et al [28] against the type V collagen antibody in order to determine the isoelectric point of the antigenic determinant.

Enzyme-Linked Immunoabsorbent Assay The standard enzyme-linked immunoabsorbent assay (ELISA) was used as described by Rennard et al [29]. Briefly, 2.0 μg of laminin, fibronectin, elastin, heparan sulfate proteoglycan, and collagens type I, IV, and V were absorbed to the bottom of 96-well microtiter plates (Dynatech) in carbonate buffer, pH 9.2, and left for at least 24 h at 4°C. Before using and between each step of the ELISA procedure, the plates were washed 3 times for 3 min with 0.15 M NaCl, 0.2 M sodium phosphate, pH 7.2, containing 0.05% Tween 20 detergent. Serial dilutions of the control or experimental antibodies from 1:5–1:2000 in a volume of 100 μl were incubated with each component for 1 h. The plates were then washed and incubated for 1 h with a species-specific peroxidase-labeled anti-IgG diluted 1:1000, washed, and revealed by the addition of orthophenylene diamine (Aldrich). The reactions were terminated with 8 M H_2SO_4 and the absorbance of the product measured in a Dynatek (Alexandria, Virginia) spectrophotometer at 405 nm.

Collagenase Treatment of Type V Collagen One milligram of lyophilized type V collagen was readily solubilized in 250 μl of 0.025 M Tris-HCl (pH 7.2), 1.8 M NaCl and dialyzed against 0.025 M Tris-HCl (pH 7.2), 0.01 M calcium acetate, 0.001 M phenylmethylsulfonyl fluoride, 0.04 M N-ethylmaleimide. Five units (25 μl) of proteinase-free collagenase (Advanced Biofactures, Type III, Lynbrook, New York) or the same amount of boiled collagenase was added and incubated at 37°C for 2 h. The reaction was halted by placing the tubes on ice and by adding 30 μl of 0.1 M EDTA. The samples were then dialyzed against 0.025 M sodium phosphate, pH 6.8, 2% SDS, 10% glycerol, 0.002% bromophenol blue (sample buffer), and made 0.1 M dithiothreitol, boiled for 2 min, and subjected to SDS-PAGE and Western blot analysis.

Immunofluorescence Fresh normal human skin from surgical sections or punch biopsies from volunteers was obtained. The skin was immediately embedded in O.C.T. cryogel (Tissue Tek, Naperville, Illinois) and quick-frozen in liquid nitrogen. Other skin samples were incubated in 1 M NaCl at 4°C for 72 h and the epidermis separated from the dermis through the lamina lucida area of the BMZ as previously described [15]. This salt-separated substrate was then embedded and frozen as above.

Both intact and salt-separated skin were used as substrates for indirect immunofluorescence (IIF) with control and experimental sera diluted 1:10–1:640 in Dulbecco's phosphate-buffered saline (PBS) as previously described [15]. In addition to immune and preimmune goat sera, immunofluorescent staining on intact and salt-separated skin was performed with: (1) a rabbit antibody to laminin (BRL Laboratories, Bethesda, Maryland) diluted 1:40, (2) an affinity-purified sheep antibody to type IV collagen that has been described elsewhere [21], (3) a murine monoclonal antibody to type IV collagen as previously reported [23], and (4) a murine monoclonal antibody (H3a) to the EBA antigen in skin [25].

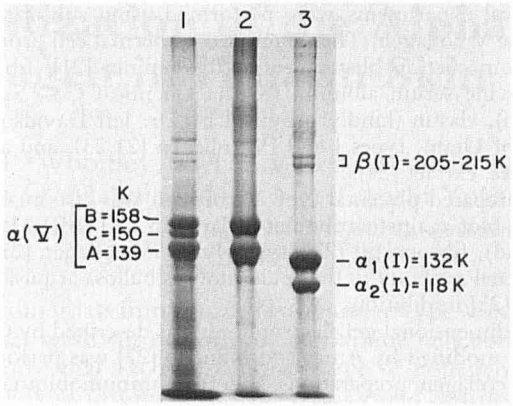

Figure 1. A Coomassie Blue stained 5% sodium dodecyl polyacrylamide slab gel of partially purified human placental type V collagen (*lane 1*), a type V collagen standard from Dr. Burgeson (*lane 2*), and human type I collagen (*lane 3*). *Lane 1* was overloaded with 110 μg of protein to emphasize the C chain of type V collagen that is found in preparations from whole placenta but is absent in preparations from amnion (*lane 2*). The type I collagen alpha chains (*lane 3*) migrate lower than the type V collagen alpha chains. The relative migrations of the bands as labeled here were calculated against commercially available molecular weight standards (Bio-Rad, Rockville Centre, New York). However, collagens are known to run anomalously high in Laemmli gels. When the relative migrations of the bands are calculated against type I collagen as a standard (a kind gift from Dr. Gerald Mechanic, University of North Carolina School of Medicine), the calculated relative migrations of the bands in this figure are: α_1 (I) = 95,000 kD, α_2 (I) = 92,000 kD, α_1 (V) = B chain = 128,000 kD, α_2 (V) = A chain = 103,000 kD, and α_3 (V) = C chain = 118,000 kD.

Immunoelectron Microscopy Indirect immunoelectron microscopy was performed using a multistep peroxidase-antiperoxidase method as previously described [30] with several modifications. Cryostat sections (10–12 μm thick) were cut using frozen intact normal human skin and salt-separated human skin substrate. The sequence of immune reactions was as follows: (1) goat antihuman type V collagen antibody diluted 1:10 and 1:20 in PBS,

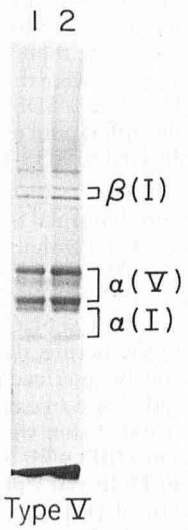

Figure 2. A silver-stained 5% sodium dodecyl polyacrylamide slab gel of the 2 type V collagen preparations routinely used in Western blots for screening antibody. Highly sensitive silver staining shows that the preparation is still contaminated with alpha and beta chains of type I collagen. The advantage of this preparation is that antibody specificity for both type I and type V collagen can be determined in the same blot.

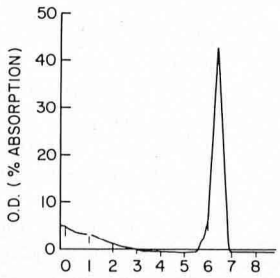

Figure 3. Unreduced type V collagen solubilized in 0.5 M acetic acid and chromatographed by high-pressure liquid chromatography on a TSK 3000 gel filtration column (*O.D.* = optical density).

(2) affinity-purified rabbit antigoat IgG antibody diluted 1:20 and 1:10 (Cappel Laboratories, Malvern, Pennsylvania), and (3) goat antihorseradish peroxidase antibody diluted 1:20 (Cappel Laboratories, Malvern, Pennsylvania). Immune reaction product was developed using Hanker-Yates solution (Polysciences, Warrington, Pennsylvania).

RESULTS

The type V collagen prepared from human placenta, subjected to SDS-PAGE on slab gels, and stained with Coomassie Blue, revealed the characteristic 3 main type V collagen chains (alpha 1 (V) 140 kD, alpha 2 (V) 160 kD, and alpha 3 (V) 154 kD), which comigrated identically with type V collagen sent to us from Dr. Burgeson, Portland, Oregon (Fig 1). However, when overloaded on the gels, it could be seen that some preparations were also contaminated with a small amount of type I collagen, which was revealed as alpha$_2$ (I) 122 kD and alpha$_1$ (I) 132 kD bands in addition to higher molecular weight type I beta chains (220 and 228 kD bands) (Fig 2). Other bands, which could not be identified, were also observed at 245 kD, 250 kD, 255 kD, and higher. These contaminating proteins were separated from the preparation by a CM-cellulose column equilibrated with 0.04 M lithium acetate, 0.03 M lithium acetate, 2.0 M urea pH 4.8, and a gradient of

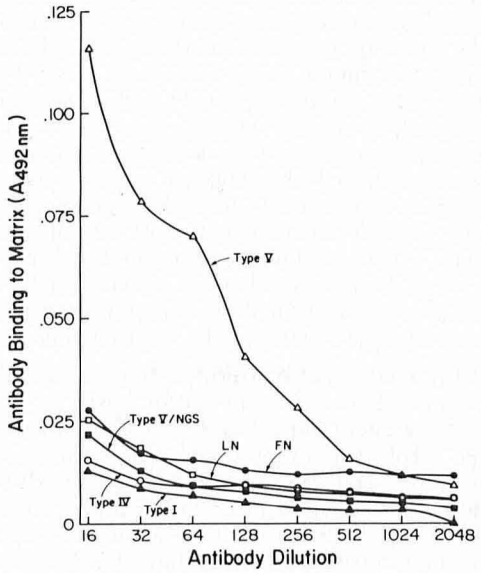

Figure 4. Enzyme-linked immunosorbent assay (ELISA) of lug of matrix molecules (*LN* = laminin, *FN* = fibronectin, type IV collagen and type V collagen) immobilized in polystyrene wells and reacted with G60 immune serum. The antibodies react with type V collagen but not the other matrix molecules. Preimmune normal goat serum (*Type V/NGS, solid squares*) and normal serum from other species (not shown) did not react with the matrix molecules tested.

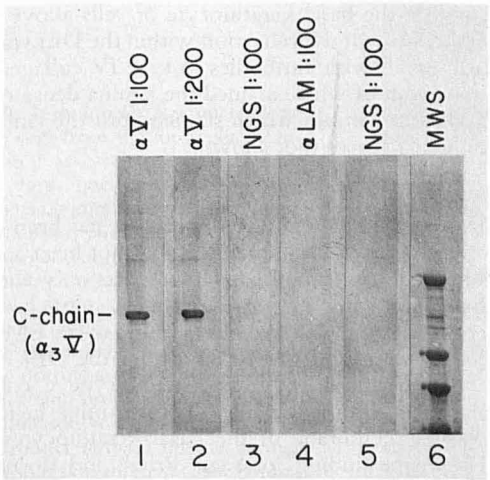

Figure 5. Western immunoblots of the type V collagen preparation shown in Fig 2 reacted with G60 serum (*lane 1* = 1:100 dilution, *lane 2* = 1:200 dilution), normal goat sera (*lanes 3 and 5*), and antibodies to laminin (*lane 4*). *Lane 6* is a molecular weight standard blotted in parallel and stained with 1% amido black. At both dilutions the G60 serum strongly labels the type V collagen C chain [α_3(V)] (M_r = 150K).

lithium chloride from 0.03 to 0.25 M at room temperature. This purified type V collagen preparation revealed only the alpha (V) chains by SDS-PAGE and showed 1 symmetrical peak when chromatographed unreduced in 0.5 M acetic acid, pH 2.5 on a TSK 3000 gel filtration column (LKB, Bromma, Sweden) by high-pressure liquid chromotography (Fig 3).

By the second month of immunization with type V alpha chains cut out of the polyacrylamide gel as described in *Materials and Methods*, 1 goat serum (G60) contained antibodies that recognized

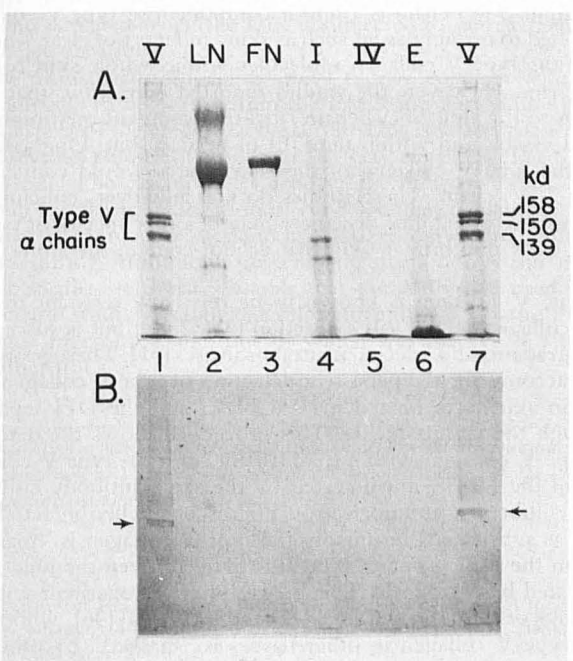

Figure 6. Western blots of type V collagen (*lanes 1 and 7*), laminin (*lane 2*), fibronectin (*lane 3*), type I collagen (*lane 4*), type IV collagen (*lane 5*), and epidermal cell proteins (*lane 6*) stained for protein with amido black (*A*) or reacted with G60 serum (*B*). The lanes containing type V collagen react with the G60 serum (*lanes 1 and 7*) which labels a 150 kD band (*arrows*).

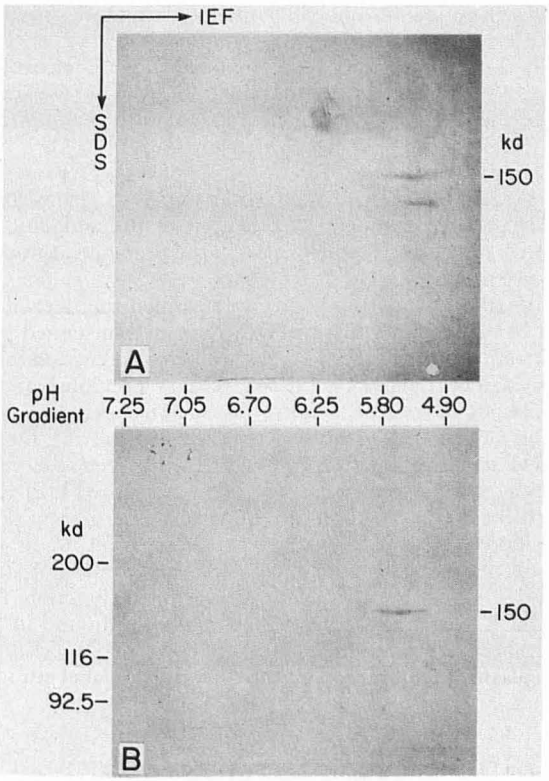

Figure 7. Two-dimensional gel electrophoresis (*A*) of partially purified type V collagen followed by Western blotting (*B*) and reacted with the G60 serum. The antigenic determinant for the antibodies in G60 serum has an isoelectric point of approximately 5.6.

the purified type V collagen preparations by an ELISA and did not label other matrix molecules (Fig 4). By Western blot analysis with the type V collagen preparations contaminated with type I collagen and other proteins, the G60 antibody reacted only with the type V collagen C chain (alpha 3 (V) chain) and none of the other proteins in the preparation (Fig 5). In identical Western blots, this antibody was found to react only with type V collagen and not with epidermal cell proteins or with other known matrix

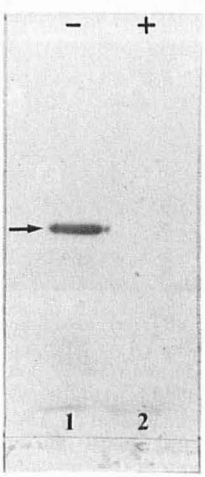

Figure 8. A Western blot of the type V collagen preparation reacted with G60 serum diluted 1:200 after treatment with boiled collagenase (*lane 1*) or 10 units of active collagenase (*lane 2*). The α_3 (V) chain (*arrow*), the target for G60 antibodies, is degraded by the bacterial collagenase.

molecules including fibronectin, laminin, elastin (not shown), and collagen types I and IV (Fig 6).

When 2-dimensional gel electrophoresis was performed on the type V collagen preparation and followed by Western blot analysis, the G60 antibody labeled a single oval band with a pI of 5.6 (Fig 7).

Collagenase treatment of the type V collagen preparation followed by SDS-PAGE and Western blot analysis against the G60 antibody showed complete degradation of the antigenic determinant by the bacterial collagenase in the presence of nonmetalloprotease inhibitors (Fig 8).

By IIF, the G60 antibody strongly stained the dermal blood vessels of intact skin while the DEJ remained unstained (Fig 9). In contrast, antibodies to laminin, type IV collagen, and the EBA antigen stained the DEJ of intact skin as previously reported [12,15,24,30]. However, when salt-separated skin was stained with the G60 antibody, both dermal vessels and the DEJ were stained. Further, the antibody bound to the dermal side of the separation and the epidermal side of the separated DEJ (Figs 9, 10). When examined with immunoelectron microscopy (Fig 10), the antibody was localized to the lower portion of the lamina lucida on the dermal side of the separation and did not stain the lamina densa or sublamina densa regions of the junction. On the epidermal side of the separation, the antibody bound in a pericellular localization around the polar aspect of the basal keratinocyte plasma membrane. The antibody did not label intracellular structures within the basal keratinocyte or cells above the basal layer. The ultrastructural localization within the DEJ was distinct from that observed with antibodies to type IV collagen and the EBA antigen (both of which stained the lamina densa region) or with antibodies to laminin which stained both the lamina densa and lamina lucida spaces (not shown).

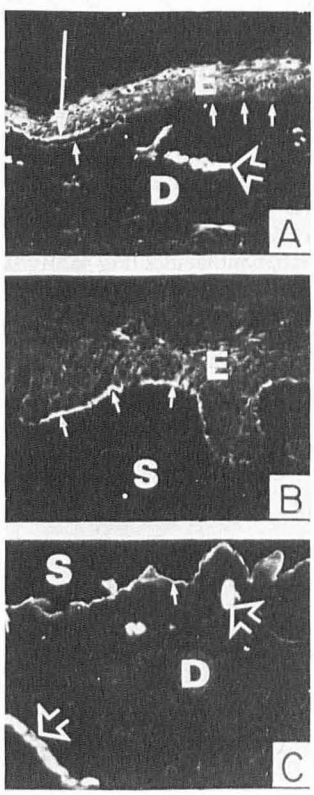

Figure 9. Indirect immunofluorescent staining by anti-type V collagen of normal human intact skin, (*A*, × 200) and human skin separated at 4°C by 1 M NaCl (*B*, epidermal roof, × 300; *C*, dermal floor, × 300) with G60 serum. The antibodies stain dermal vessels (*hollow arrows, A and C*) but not the DEJ of intact skin (*A, series of 3 small solid arrows*). In the same figure, an area of fortuitously split junction is seen and antibody labeling on each side of the split (*long arrow* points to epidermal staining and *short arrow* to dermal staining. In (*B*) and (*C*), human skin has been deliberately split at the junction with a NaCl treatment and the G60 serum stains the polar aspect of the separated epidermis (*B*) and the basement membrane on the dermal side of the separation (*C*). (*E* = epidermis, *D* = dermis, *S* = separation space.)

DISCUSSION

The localization of type V collagen in tissues has been disputed, and the localization within human skin has not been conclusive. In this study, using a goat antibody that reacts only with type V collagen by ELISA and Western blot analysis, we have shown that the C chain of type V collagen is localized to the BMZ around dermal blood vessels and the BMZ within the DEJ of human skin. The ultrastructural localization of the type V collagen C-chain [alpha $(V)_3$] within the BMZ is the lamina lucida region and the plasma membrane of the basal keratinocytes. This is consistent with the findings of Sage, Pritzl, and Bornstein [31] who found the alpha C chain of type V collagen associated with basement membranes [31,32], but did not do immunoelectron microscopy on human skin to show its ultrastructural localization. It should be emphasized that the alpha 3 (V) chain or "C" chain of type V collagen is not found in many species of type V collagen including that isolated from human amnion without placental tissue [33], chicken gizzard [11], chick embryo muscle tissue [34], chicken bone [35], corneal fibroblasts [36], chick tendon fibroblasts, and hamster lung cells [37]. Further, the organization of type V collagen chains within tissues has been unclear [31]. *Homopolymeric* forms such as [alpha 1 $(V)]_3$ and [alpha 2 $(V)]_3$ [33,38,39] and *heteropolymeric* forms such as [alpha 1 $(V)]_2$ alpha 2 (V) [31,34,40,41] have been proposed. In most tissues and cell cultures, the predominant form of type V collagen appears likely to be [alpha 1 $(V)]_2$ alpha 2 (V) [31,34,40]. However, in uterus [42], placenta villi [16,43,44], synovial membrane [16,41,44,45], mucosa, and skin [16,41,44,45], a third minor but distinct chain, alpha 3 (V) or the "C" chain is found when type V collagen is isolated. In these tissues, the type V collagen contains alpha 2 (V) chains in association with alpha 1 (V) and/or alpha 3 (V) chains [31]. The alpha 3 (V) chain is a minor component of type V collagen restricted to certain tissues such as skin, and it is not clear whether all of the type V collagen molecules within whole skin contain this chain. However, the studies reported here show that antibodies to the alpha 3 (V) chain label the basement membranes in blood vessels and within the DEJ of human skin. One assumes that the type V collagen of these structures would contain the alpha 3 (V) chain. These studies do not, however, conclusively rule out the possibility that there may be other species of type V collagen within human skin that do not contain the alpha 3 (V) chain.

Type V collagen is known to be relatively resistant to both skin collagenase and salt extraction [31,32,46], but is susceptible to degradation by selected neutral proteases [31]. These properties may account for the persistent detection of type V collagen after human skin is incubated in 1 M NaCl and the DEJ separated through the lamina lucida. This procedure allows the detection of type V collagen within the DEJ. In contrast, type V collagen within the DEJ is not detected by the same antibody either by IIF or indirect immunoelectron microscopy when intact skin is used as substrate. Presumably the type V collagen is "masked" within the DEJ of intact skin and revealed when the junction is separated by 1 M NaCl. This presumption is consistent with the findings of Linsenmayer et al [11] and Fitch et al [46], who found that type V collagen in other tissues is "masked" by other extracellular matrix molecules and could be "revealed" by a variety of techniques including tissue incubations in dilute acidic acid or collagenase and by varying the temperature of the incubations [11,46]. Fitch et al [46] found that immunofluorescent staining of type V collagen was masked at 37°C and then revealed by lowering the temperature to 0°C.

Large matrix molecules within the junction are known to have

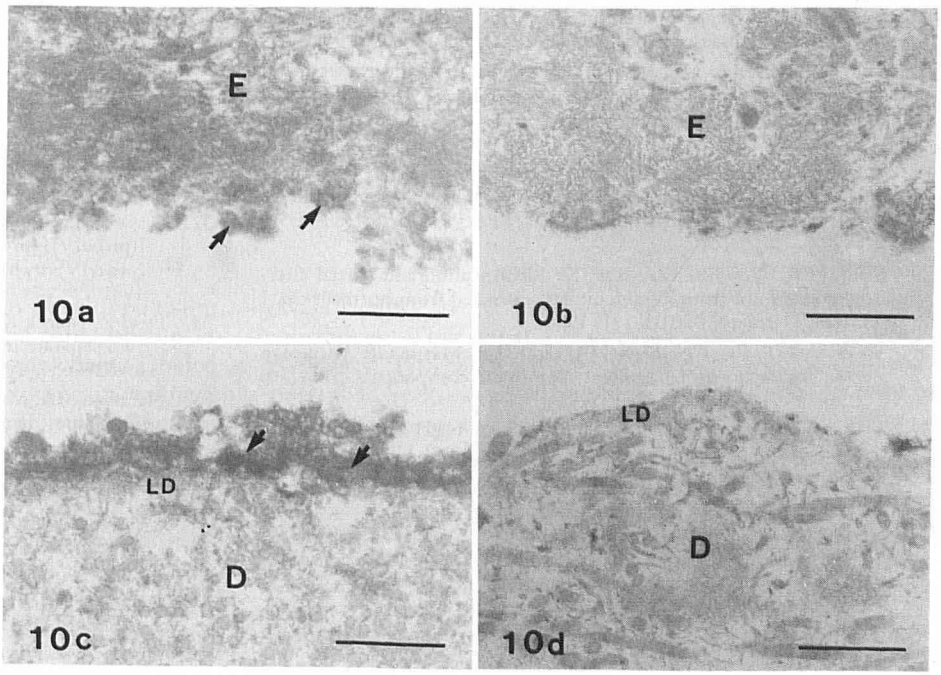

Figure 10. Immunoelectron microscopy of human skin from the hip, split by incubation in 1 M NaCl and stained with antibody. Immunoelectron micrographs are shown using goat anti-type V collagen antibody (*a and c*) compared with normal goat serum control (*b and d*). *a*, Demonstrates immune reaction product lining the basal surface of the epidermis (*E*) and in the previous lamina lucida (*arrows*). *b*, Shows the epidermal (*E*) side of the split skin, using normal goat serum as control. Immune reaction product is absent at the previous junction zone. *c*, Demonstrates reaction product (*arrows*) on the dermal side (*D*) of split skin adjacent to the lamina densa (*LD*). *d*, Shows the dermal side (*D*) of the split skin using goat serum control. Reaction is absent in the junction zone. *Calibration bars* = 0.5 μm.

specific affinities for each other [21], and the molecular organization of this dense region has been suggested by rotary shadowing studies with purified components alone and in various combinations [47,48]. The region is highly anionic due to the presence of heparan sulfate proteoglycan both within the DEJ and the plasma membrane of the apposing cells [49,50]. In light of the organization and chemical properties of the DEJ and the specific alignments among junctional components that are required presumably to keep the structure intact, it is perhaps not surprising that one or more of the components might be masked in situ and revealed when the DEJ is subjected to salt extraction or other perturbations.

The ultrastructural localization of type V collagen within the DEJ appears to be both within the BMZ and pericellular on the polar aspect of the epidermal basal cell. This is in accordance with the previous studies of Roll et al [7], Gay et al [13], and Sage et al [31,32]. The discrepancies reported in previous reports concerning the tissue localization of type V collagen may have been due to variations in tissues and in the handling of the tissue substrates during immunofluorescent and immunoelectron microscopic studies. It is also possible that the antibodies used in the discrepant studies recognized different epitopes on the type V collagen macromolecule, one of which was masked in situ and the other not.

We would like to thank Dr. Robert Burgeson for his thoughtful advice and sharing of reagents such as a type V collagen control. We also thank Ms. Jo Webb for her technical assistance.

REFERENCES

1. Burgeson RE, Adli FA, Kaitila II, Hollister DW: Fetal membrane collagens: identification of two new collagen alpha chains. Proc Natl Acad Sci USA 73:2579–2583, 1976
2. Chung E, Rhodes RK, Miller EJ: Isolation of three collagenous components of probably basement membrane origin from several tissues. Biochem Biophys Res Commun 71:1167–1174, 1976
3. Rhodes RK, Miller EJ: Evidence for the existence of an α1 (V) α2 (V) α3 (V) collagen molecule in human placental tissue. Coll Relat Res 1:337–343, 1981
4. Modesti A, Kalebic T, Scarpa S, Togo S, Grotendorst G, Liotta LA, Triche TJ: Type V collagen in human amnion is a 12 nm fibrillar component of the pericellular interstitium. Eur J Cell Biol 35:246–255, 1984
5. Duance VC, Restall DJ, Beard H, Bourne FJ, Bailey AJ: The localization of three collagen types in skeletal muscle. FEBS Lett 79:248–252, 1977
6. Bailey AJ, Shellswell GB, Duance VC: Identification and change of collagen types in differentiating myoblasts and developing chick muscle. Nature 278:67–69, 1979
7. Roll FJ, Madri JA, Albert J, Furthmayr H: Codistribution of collagen types IV and AB_2 in basement membranes and mesangium of the kidney. An immunoferritin study of ultrathin frozen sections. J Cell Biol 85:597–616, 1980
8. Gay SA, Martinez-Hernandez A, Rhodes RK, Miller EJ: The collagenous exocytoskeleton of smooth muscle cells. Coll Relat Res 1:337–384, 1981
9. Madri JA, Dreyer B, Pitlick FA, Furthmayr H: The collagenous components of the subendothelium. Lab Invest 43:303–315, 1980
10. Sano J, Fujiwara S, Sato S, Ishizak M, Sugisaki Y, Yajima G, Naga Y: AB (type V) and basement membrane (type IV) collagens in bovine lung parenchyma: electron microscopic localization by the peroxidase-labeled antibody method. Biomed Res 2:20–29, 1981
11. Linsenmayer TF, Fitch JM, Schmid TM, Zak NB, Gibney E, Sanderson RD, Mayne R: Monoclonal antibodies against chicken type V collagen: production, specificity, and use for immunocytochemical localization in embryonic cornea and other organs. J Cell Biol 96:124–132, 1983
12. Stenn KS, Madri JA, Roll FJ: Migrating epidermis produces AB_2 collagen and requires continuous collagen synthesis for movement. Nature 277:229–232, 1979
13. Gay S, Kresina TF, Gay R, Miller EJ, Montes LF: Immunohistochemical demonstration of basement membrane collagen in normal human skin and in psoriasis. J Cutan Pathol 6:91–95, 1979
14. Konomi H, Hayashi T, Nakayasu K, Arima M: Localization of type V collagen and type IV collagen in human cornea, lung and skin. Am J Pathol 116:417–426, 1984
15. Woodley DT, Sauder D, Talley MJ, Silver M, Grotendorst G, Qwarnstrom E: Localization of basement membrane compounds after dermal-epidermal junction separation. J Invest Dermatol 81:149–152, 1983
16. Sage H, Bornstein P: Characterization of a novel collagen chain in human placenta and its relation to AB collagen. Biochemistry 18:3815–3821, 1979

17. Laemmli U: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685, 1970
18. Garvey JS, Cremer NE, Sussdorf DH: Separation and preservation of serum, in Methods in Immunology. Edited by JS Garvey, WE Cremer, DH Sussdorf. Reading, MA, WA Benjamin Inc, 1977, pp 36–38
19. Towbin H, Staehelin T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76:4350–4354, 1979
20. Stanley JR, Woodley DT, Katz SI: Identification and partial characterization of pemphigoid antigen extracted from normal skin. J Invest Dermatol 82:108–111, 1984
21. Woodley DT, Rao CN, Hassel JR, Liotta LA, Martin GR, Kleinman HK: Interactions of basement membrane components. Biochim Biophys Acta 761:278–283, 1983
22. O'Keefe EJ, Payne RE, Russell N, Woodley DT: Spreading and enhanced motility of human keratinocytes of fibronectin. J Invest Dermatol 85:125–130, 1985
23. Scheinman JI, Tsai C: Monoclonal antibody to type IV collagen with selective basement membrane localization. Lab Invest 50:101–112, 1984
24. Woodley DT, Briggaman RA, O'Keefe EJ, Inman AO, Queen LL, Gammon WR: Identification of the epidermolysis bullosa acquisita antigen: a normal component of human skin basement membrane. N Engl J Med 310:1007–1013, 1984
25. Paller AS, Queen LL, Woodley DT, O'Keefe EJ, Gammon WR, Briggaman RA: A mouse monoclonal antibody against a newly discovered basement membrane component, the epidermolysis bullosa acquisita antigen. J Invest Dermatol 84:215–217, 1985
26. O'Farrell PH: High resolution two-dimensional electrophoresis of proteins. J Biol Chem 250:4007–4021, 1975
27. Ames GF, Nikaido K: Two dimensional gel electrophoresis of membrane proteins. Biochemistry 15:616–623, 1976
28. Koulu L, Kusumi A, Steinberg MS, Klaus-Kovtun V, Stanley JR: Human autoantibodies against a desmosomal core protein in pemphigus foliaceus. J Exp Med 160:1509–1519, 1984
29. Rennard SI, Berg R, Martin GR, Foidart JM, Robey PG: Enzyme-linked immunoassay (ELISA) for connective tissue components. Anal Biochem 104:205–214, 1982
30. Yaoita H, Briggaman RA, Lawley TJ, Provost TT, Katz SI: Epidermolysis bullosa acquisita: ultrastructural and immunological studies. J Invest Dermatol 76:288–292, 1981
31. Sage H, Pritzl P, Bornstein P: Susceptibility of type V collagen to neutral proteases: evidence that the major molecular species is a thrombin-sensitive heteropolymer, $[\alpha 1\,(V)]_2\,\alpha 2\,(V)$. Biochemistry 20:3778–3784, 1981
32. Sage H: Collagens of basement membranes. J Invest Dermatol 79 (suppl):51s–59s, 1982
33. Rhodes RK, Miller EJ: Physiochemical characterization and molecular organization of the collagen A and B chains. Biochemistry 17:3442–3448, 1978
34. Kumamoto CA, Fessler JH: Biosynthesis of A, B procollagen. Proc Natl Acad Sci USA 77:6434–6438, 1980
35. Brock DL, Madri J, Eikenberry EF, Brodsky B: Characterization of the tissue form of type V collagen from chick bone. J Biol Chem 260:555–562, 1985
36. Poschl A, Von der Mark K: Synthesis of type V collagen by chick corneal fibroblasts in vivo and in vitro. FEBS Lett 115:100–104, 1980
37. Fessler LI, Robinson WJ, Fessler JH: Biosynthesis of procollagen $[(\text{pro}\,\alpha\text{IV})_2\,(\text{pro}\,\alpha 2\text{V})]$ by chick tendon fibroblasts and procollagen $(\text{pro}\,\alpha 1\text{V})_3$ by hamster lung cell cultures. J Biol Chem 256:9646–9651, 1981
38. Deyl Z, Macek K, Adam M: Collagen αA and αB chains constitute two separate molecular species. Biochem Biophys Res Commun 89:627–634, 1979
39. Haralson MA, Mitchell WM, Rhodes RK, Kresina TF, Gay R, Miller EJ: Chinese hamster lung cells synthesize and confine to the cellular domain a collagen composed solely of B chains. Proc Natl Acad Sci USA 77:5206–5210, 1980
40. Bentz H, Bächinger HP, Glanville R, Kuhn K: Physical evidence for the assembly of A and B chains in human placental collagen in a single triple helix. Eur J Biochem 92:563–569, 1978
41. Elstow SF, Weiss JB: Extraction, isolation and characterization of neutral salt soluble type V collagen from fetal calf stain. Coll Relat Res 3:181–194, 1983
42. Abedin MZ, Ayad S, Weis JB: Type V collagen: the presence of appreciable amounts of α3 (V) chain in uterus. Biochem Biophys Res Commun 102:1237–1245, 1981
43. Ayad S, Abedin MZ, Weis JB: Non-basement membrane collagen A, B and C α-chains: types V and VI collagen. Biochem Soc Trans 8:324–325, 1980
44. Brown RA, Weis JB: Type V collagen: possible shared identity of αA, αB and αC chains. FEBS Lett 106:71–75, 1979
45. Brown RA, Shuttleworth A, Weis JB: Three new α-chains of collagen from a non-basement membrane source. Biochem Biophys Res Commun 80:866–872, 1978
46. Fitch JM, Gross J, Mayne R, Johnson-Wirt B, Linsenmayer TF: Organization of collagen types I and V in the embryonic chicken cornea: monoclonal antibody studies. Proc Natl Acad Sci USA 81:2791–2795, 1984
47. Laurie GW, Kleinman HK, Hassell JR, Martin GR, Feldman RJ: Basement membrane organizations suggested by combinations of laminin and heparan sulfate proteoglycan binding sites with the "open network" and "polygonal" models of type IV collagen. J Cell Biol 101:259a, 1985
48. Yurchenco PO, Furthmayr H: Self assembly of basement membrane collagen. Biochemistry 23:1839–1849, 1985
49. Hassell JR, Gehron-Robey P, Barrach H-J, Wilcek J, Rennard SI, Martin GR: Isolation of a heparan sulfate-containing proteoglycan from basement membrane. Proc Natl Acad Sci USA 77:4494–4498, 1980
50. Kjellen L, Oldberg A, Hook M: Cell-surface heparan sulfate. J Biol Chem 255:10,407–410,413, 1980